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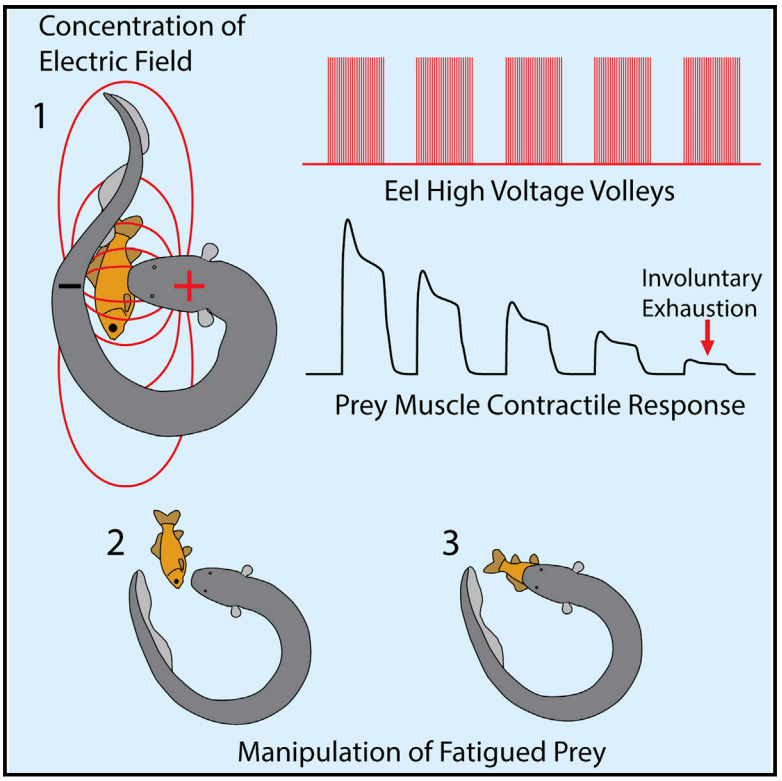
Electric Eels Concentrate Their Electric Field to Induce Involuntary Fatigue in Struggling Prey

Graphical Abstract



Highlights

- Electric eels leverage the physics of dipole fields to maximally electrify prey
- Eels sandwich struggling prey between positive and negative electrical poles
- Subsequent volleys of amplified discharges ensure prey muscles become fatigued

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In Brief

Catania reports that electric eels have found a way to greatly amplify the effect of their high-voltage weaponry and that they use this to cause involuntary fatigue in struggling prey.

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Electric Eels Concentrate Their Electric Field to Induce Involuntary Fatigue in Struggling Prey

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SUMMARY

Nature is replete with predator venoms that immobilize prey by targeting ion channels. Electric eels (*Electrophorus electricus*) take a different tactic to accomplish the same end. Striking eels emit electricity in volleys of 1 ms, high-voltage pulses. Each pulse is capable of activating prey motor neuron efferents, and hence muscles. In a typical attack, eel discharges cause brief, immobilizing tetanus, allowing eels to swallow small prey almost immediately. Here I show that when eels struggle with large prey or fish held precariously, they commonly curl to bring their own tail to the opposite side of prey, sandwiching it between the two poles of their powerful electric organ. They then deliver volleys of high-voltage pulses. Shortly thereafter, eels juggle prey into a favorable position for swallowing. Recordings from electrodes placed within prey items show that this curling behavior at least doubles the field strength within shocked prey, most likely ensuring reliable activation of the majority of prey motor neurons. Simulated pulse trains, or pulses from an eel-triggered stimulator, applied to a prey muscle preparations result in profound muscle fatigue and loss of contractile force. Consistent with this result, video recordings show that formerly struggling prey are temporarily immobile after this form of attack, allowing the manipulation of prey that might otherwise escape. These results reveal a unique use of electric organs to a unique end; eels superimpose electric fields from two poles, ensuring maximal remote activation of prey efferents that blocks subsequent prey movement by inducing involuntary muscle fatigue.

INTRODUCTION

The electric eel (*Electrophorus electricus*) stands out as a formidable predator by virtue of its uncommon electrical weaponry and unique hunting strategies. When they strike, electric eels generate hundreds of volts of electricity, delivered in 1 ms pulses, at rates that approach 500 Hz [1–3]. The attack volley of the eel generally activates the motor neurons in nearby prey [3, 4], such that each electrical pulse is translated into a prey motor neuron action potential and inevitably, shortly thereafter, into a muscle contraction. As a result, the function of the eel's attack volley is analogous to a TASER's [5]. High rates of discharge cause high rates of muscle contraction, resulting in immobilizing tetanus in prey (and in potential predators, including humans [6]). Eels take advantage of this brief period of immobility to seize prey, which are then swallowed whole.

The description above provides an example of eel predation typically observed when providing feeder goldfish to a large eel in an aquarium. But electric eels live in the Amazon and are surrounded by the greatest diversity of fish species in the world [7]. Prey are likely to vary in size, shape, and skin resistance and may sport defensive spines. Moreover, there are little data on eels' natural diets, and eels in captivity attack and eat crayfish (see the supplemental movies). It is unlikely these are mistaken for fish. These observations suggest that natural eel diets include diverse and sometimes challenging prey. What happens when an eel struggles with large prey that may not be easily subdued or swallowed? Or when juvenile eels attack?

In this case, eels have an option not available to any other strongly electric species. Because the eel's electric organ spans most of its long, flexible body, the positive and negative poles (head and tail, respectively) are widely separated in space. A typical attack is "monopolar," with the positive head providing the predominate influence on the local electric field near the prey. An electric eel could theoretically double the strength of the electric field experienced by prey if it brought its tail (the negative pole) around and behind the prey. Here I report that this is a common behavior used by eels to subdue struggling prey that have been captured but must be repositioned for swallowing. The consequences of this curling behavior for the resulting electrical field experienced by the prey were investigated using electrodes inserted into dead (pithed) fish with viable muscles that were attacked by eels. The effect of this behavior on prey muscles was explored by simulating the eel's discharge pattern and delivering it to prey muscle preparations, or alternatively by configuring a stimulator to be driven in real-time by an eel as it curled around a prey item and delivered shocks. The results reveal that electric eels can inactivate prey muscles by inducing high rates of involuntary activation of prey efferents, producing temporary, but debilitating, muscle fatigue. This gives eels a window of opportunity to manipulate prey for swallowing—a period during which prey have their last, fleeting opportunity to escape as they are briefly released and repositioned.

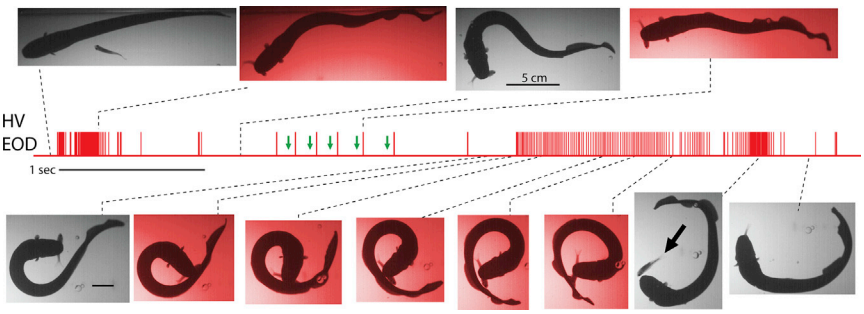

Figure 1. Example of a Small, Juvenile Eel Curling for a Dipole Attack before Manipulating Prey

Frames are captured from high-speed video (see Movie S1, clip 1). Red colorized frames corresponded to a high-voltage discharge. Middle trace shows the relative timing of high-voltage discharges as red tick marks, and the time points for each frame are indicated with a dotted line. Green arrows indicate the time of a voluntary fish movement as seen on the high-speed video. Each fish movement was immediately followed by an eel discharge and eventually the curling behavior. Note that less than 500 ms after the curl, the eel has completely released the fish (arrow) while repositioning for a head-first swallow.

RESULTS

Context and Description of the Dipole Attack

Figure 1 illustrates the stereotyped sequence of events that usually occurs during a curling attack by a small, juvenile eel. The behavior is associated with prey handing after capture, and it predominantly occurs when a prey item is held such that it cannot be swallowed until it has been repositioned. Movie S1, clip 1 shows this trial at 250 frames per second (fps), filmed in silhouette with 940 nm illumination. The eel partially inactivated the prey with its initial attack and high-voltage discharge but captured the fish lengthwise. This posed a challenge for the eel, which had to briefly release its firm grip on the struggling fish in order to swallow it headfirst. Active fish frequently escape at this point (Movie S2). Before repositioning the fish, the eel curled to bring its head and tail together with the fish sandwiched in between. It then gave off a long volley of high-voltage pulses at approximately 100 Hz. The eel then released the immobilized fish and swallowed it head first in conjunction with high-voltage discharges.

The behavior just described is not simply one extreme in a continuous spectrum of eel movements when handling prey. Rather, it consists of a unique sequence of behaviors performed in a particular context by every eel that was investigated or observed. Figure 2 illustrates the sequence and highlights different phases of the behavior. The behavior was very commonly observed in juvenile electric eels handling any fish (Movie S1). It was also common in intermediate sized eels handling large fish. It was less common in large electric eels handling fish, but it was easily elicited in even the largest eel by presenting it with a challenging prey item, such a large crayfish that had to be repositioned multiple times before swallowing (Movie S3), or by mimicking this situation as described and shown in later sections.

The remainder of this study is aimed at addressing the following questions: What is the result of the curling behavior on the electric field experienced by a prey item? What is the effect of this tactic on prey behavior? And finally, in light of the previous questions, what is the function of the behavior?

Theoretical Effect of Curling on Electrical Field Experienced by Prey

Electrocytes (non-contractile myocytes that generate electricity [8]) come in a wide range of morphologies, are distributed in different locations in different species, and generate a diversity of waveforms [9–18]. Eels have among the simplest of electrical discharges; each is monophasic and head positive [19]. A long "main" electric organ provides the majority of the high voltage discharge and is insulated along its length such that the current source and sink are widely separated in space [19].

The fields generated by different configurations of electrocytes have been measured and modeled [20–29] in numerous investigations since the seminal description of active electrolocation by Lissmann [30]. As a first approximation, the electric field can usually be modeled as a dipole field surrounding the fish [31]. Empirical measures have revealed some important deviations from the predictions of a dipole configuration. For example, it has been found that close to an electric fish, the poles do not act as point sources (see [31] for a review), as generally illustrated for dipoles in classical electrostatics. Rather, the low internal resistance of the fish body distributes the local current source to more closely resemble a line charge. Field strength falls more slowly with distance (d) from an idealized line charge ($1 / d$) compared to a point source ($1 / d^2$). Despite this limitation, a dipole model, consisting of two point sources is used here as an approximation for electric eels and discussion of their behavior.

Figure 3A shows a schematic illustration of a dipole electric field surrounding an eel during its high-voltage discharge, with poles at the head and at the approximate end of the main electric organ in the tail. A seemingly inescapable conclusion from any similar field configuration is that bringing the negative pole close to the positive head will intensify the electric field in between the head and tail (Figure 3B). It is not possible to test this hypothesis with electrodes attached to the aquarium walls. Rather, the electrodes must measure potential differences in the space between the head and tail.

Measured Effect of Curling on Electrical Field Experienced by Prey

It was fortuitous that the very eel behavior being investigated lent itself to an experimental paradigm for measuring electrical potential differences within prey as they were held between the head and tail (Movie S4). As outlined in Figures 1 and 2, the stimulus for eliciting the curling behavior is a captured, struggling prey item that cannot be immediately swallowed. With moldable plastic and thin, insulated motor wire, a dual electrode configuration was designed that allowed a pithed fish (with viable

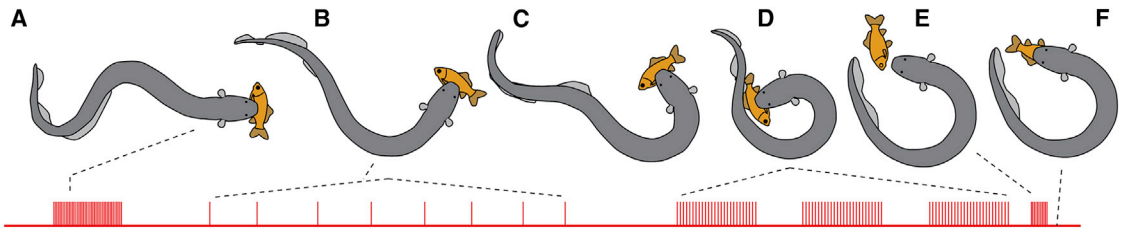

Figure 2. Schematic Illustration of the Sequence of Events during a Typical Dipole Attack

(A) The fish is captured in conjunction with a high-frequency, high-voltage volley (~400 Hz).

(B) The fish cannot be swallowed without being re-positioned. Additionally, the fish is struggling, and each discrete fish movement elicits a brief discharge by the eel.

(C and D) The eel curls to bring its head and tail into opposition and delivers a series of high-voltage volleys, each at ~100 Hz with variable duration.

(E and F) Less than 500 ms after the dipole attack, the fish is released and repositioned for a head-first swallow in conjunction with high-voltage discharges. See Movies S1, S2, S3, and S4 for numerous examples.

muscles) containing electrodes to be presented to the eel. The preparation could not be swallowed (such a situation would probably be common for a wild eel that had captured a fish, with dorsal spines, tail first). Manual vibration of the wire simulated prey struggling and readily and repeatedly induced the curling behavior and corresponding high-voltage discharges. As previously described, prey are held tightly until after the curling behavior is complete. Similarly, the pithed-fish and corresponding electrode were held tightly by the eel and remained in the same relative position for long periods of recording during the trials.

Before the results from this paradigm are described, it is important to briefly note that eels do not modulate the amplitude of their high-voltage output during a volley (but see [32] for longer-term, hormonal regulation of gymnotiform waveforms). The simple neural control circuitry for the eel's electric organs ensures that every electrocyte in all three of the eel's electric organs participates in the high-voltage output [33–35]. It is possible that fatigue causes some decrement in high-voltage output over time during a volley. However, there is no mechanism for the opposite to occur; eels cannot increase the total power of their high-voltage output over time during a volley [33–35].

Figures 4 and 5 illustrate the dramatic effect of eel curling on the electrical potential, and hence electric field, experienced by the prey item sandwiched between electrical poles. In these trials, the electrodes were held by the eel in a line approximately parallel to the predicted [21, 30] field lines (normal to the predicted isopotential lines) for the corresponding electric field generated during each discharge. Similar results were obtained for ten trials for each of two different electric eels of intermediate size. Fewer, but similar, results were obtained from very small juvenile eels (Figure S1). Results for different trials were not combined because all relative measurements depend on the specific location and orientation of the electrodes held by the eel, and this varied between trials. However, within a trial, the measured potential could be compared for periods during which the preparation was held tightly by the eel. These time periods are color coded in Figures 4 and 5A–5C. Comparison of the average voltage for the curled versus uncurled condition reveal an obvious and significant difference in amplitude. There is no overlap in the voltage distributions for discharges from the two conditions (see the figure legends for statistics). Similar results were obtained across trials (see also Figure S1). A positive relationship between voltage and decreasing tail distance is suggested from the data in Figures 4 and 5A. This is more clearly borne out in Figures 5F and 5G, which provide an example of a "double curl" and corresponding high-voltage discharges throughout the full range of eel body positions. This behavior was common and could be induced in the experimental paradigm by manual simulation of continuous prey twitching through the electrode wire. Together, these data confirm that electric eels can greatly increase electric field strength within prey using the second pole of their electric organ. What effect does this electrical tactic have on prey?

Effect of High Frequencies of Continuous Efferent Activation on Prey Muscles

Previous investigations have shown that eel high-voltage discharges are capable of activating the motor neurons, and hence the muscles, of nearby prey [3, 4]. Intensifying the electric field by sandwiching prey between the two poles of the electric organ would most likely ensure reliable activation of the majority of prey efferents in diverse prey species of variable skin resistance. Two different approaches were used to investigate the effect of such stimulation trains on prey muscles. First, tension was measured in a pithed fish (Figure 6A) that was attached to a force transducer and stimulated with a Grass SD9 stimulator, in a manner similar to the high-voltage volleys emitted by electric eels. In this paradigm, tension was first measured for discrete pulses (Figure 6B), followed by five consecutive volleys of 500 ms stimulation at 100 Hz. This was followed, after a 500 ms delay, by a single discrete pulse in order to assay muscle function at a time delay similar to eel prey release while handling (e.g., Figure 2E). Figure 6C shows the summed results of this treatment on post-volley contractile force for ten trials in ten different fish preparations. The mean contractile force dropped to a small fraction of its former value (Figure 6C, red bar). Finally, after a period of 30 s, the preparation recovered a large proportion of contractile force (Figure 6C, black bars). When the same experiment was repeated but the stimulator voltage was halved, the attenuation of subsequent contractile force was substantially less (Figure S1).

The experimental paradigm described above was repeated for a crayfish tail preparation. The effect of five volleys resulted in a less dramatic attenuation of contractile force. However,

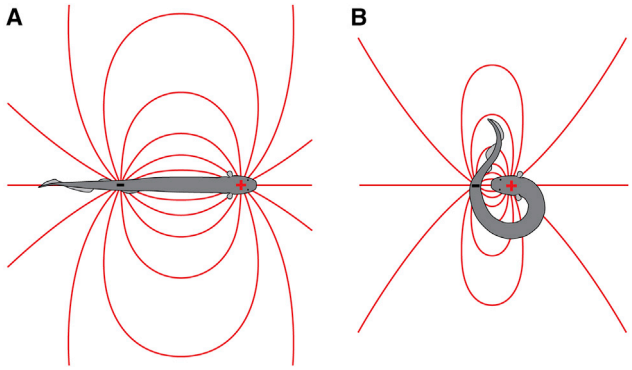

Figure 3. Schematic Illustration of a Dipole Field Surrounding an Electric Eel and Its Change in Configuration after the Eel Has Brought the Two Poles Close Together

(A) Dipole field surrounding an electric eel in a linear configuration. Lines indicate electric field lines (a positive test charge would experience a force tangent to the line at any point—in the direction of the negative pole). Equipotential lines are not illustrated but would be normal to the field lines. The (arbitrary) density of field lines reflects the intensity of the electric field.

(B) The intensity of the electric filed has been maximized between the mouth and tail by curling. The actual electric field generated by an eel would most likely diverge from this idealized schematic, for example by having more distributed sources of field lines at the head and tail.

extending the stimulation to include ten volleys had a comparable effect, as illustrated in Figures 6E and 6F. This greater number of volleys was well within the range exhibited by eels attacking both real prey and electrode preparations.

For more accurate and direct simulation of the effect of eel discharges on prey muscles, the pulse trains produced by an eel curling around a pithed fish-electrode preparation were used to trigger the stimulator in real time. The stimulator leads were in turn attached to a separate pithed-fish, or crayfish tail preparation, attached to a force transducer in an adjacent aquarium. It should be kept in mind, that this experiment addresses only the frequency (rate) of muscle stimulation. Stimulator amplitude remained constant throughout, at a voltage that produced a smaller potential difference within the fish preparation than was produced by the eel's curling behavior (see the Experimental Procedures). Nevertheless, the amplitude of the eel's discharge, as measured within the prey item, is illustrated for these trials (Figure 7, blue traces) to provide additional examples of the concentrating effect of the eel's curl on electric field strength.

Each pulse of the eel generated a pulse from the stimulator (Figure 7B), and fish or crayfish tail tension was simultaneously monitored (Figures 7C and 7D). The results of this experiment confirm the effect of volleys of high-frequency electrical stimulation on prey muscles. The high rates of continuous efferent activation triggered by the eel in the curled configuration resulted in rapid attenuation of contractile force as measured by fish whole-body tension or crayfish tail contraction. Two examples of each muscle preparation are illustrated in Figures 7E–7H.

DISCUSSION

Electric eels already stand out as the most powerful electrogenic species, capable of generating over 600 V [36]. The amplifying effect of their curling behavior at least doubles the effective power of their discharge through prey compared to an eel in a linear position. But curling may provide even greater relative amplification, as suggested from the recording data (Figures 4 and 5). This is most likely possible because the rostral pole of the eel's electric organ is located behind the head and viscera, at a fixed distance from prey held in the mouth. The caudal pole is not constrained by this anatomical limitation, and may be brought into nearly direct contact with prey—potentially having a greater effect than the rostral pole (to which its effect is added).

Behavioral precursors that might have existed in ancestral species and been selected to give rise to full curling are obvious. Deviations from a linear body configuration cause variations in the electric field configuration [37]. This general effect has been suggested to explain the unusual body plans and swimming behaviors of weakly electric fish that must maintain an undistorted field to efficiently sense their surroundings [38]. In the case of eels struggling with prey, many arbitrary movements that brought the tail closer to the head would distort the electric field in a manner that increased field strength near the mouth. This is evident in the measurements made in the present study as the eels curled and uncurled (e.g., partially curled configurations).

Even the smallest (10 cm) eels curled around captured prey. The utility of amplifying field strength would seem greatest for small eels, which may not have sufficient power to reliably activate prey efferents. The frequent curling behavior exhibited by juvenile electric eels raises the possibility that presumably smaller, ancestral eels relied on this strategy. But it seems likely that even the largest eels may frequently use this strategy under more natural circumstances. This possibility is suggested by observations of a large eel handling crayfish (Movie S3). In these cases, the duration of the curling behavior may be dramatically increased, lasting over 50 s, during which the prey's limbs repeatedly contract during each volley. This is not to suggest that crayfish are common prey for electric eels. Rather, they serve as a convenient proxy for unknown but diverse prey in the Amazon.

Given the exceptional power of the electric eel's discharge, one might wonder why any amplification is needed. Slow-motion analysis of Movie S3, showing an attack on a crayfish, provides some insight. This large eel's initial high-voltage volley and attack did not cancel the crayfish escape response. A frame captured from this time point shows a classic lateral giant escape response by the crayfish and a missed suction feeding strike by the eel (Figure S2). It is unlikely that these initial crayfish movements were the result of arbitrary activation of the crayfish musculature, as they involved the subset of tail segments that are specifically appropriate for avoiding a rearward attack [39, 40]. Although the eel was able to subsequently capture the crayfish, the conclusion is that some prey are, literally, more resistant to eel discharges than others. Note in this regard that electric fish, thought to be eel prey [2, 13], may have a particularly resistive epidermis [30]. Curling to bring the second pole around would ensure maximal stimulation of muscles in resistive prey, at little cost to the eel compared to discharging in a linear position.

The effect of multiple, high-frequency activation trains on prey muscles is predictable. It inevitably causes rapid attenuation of

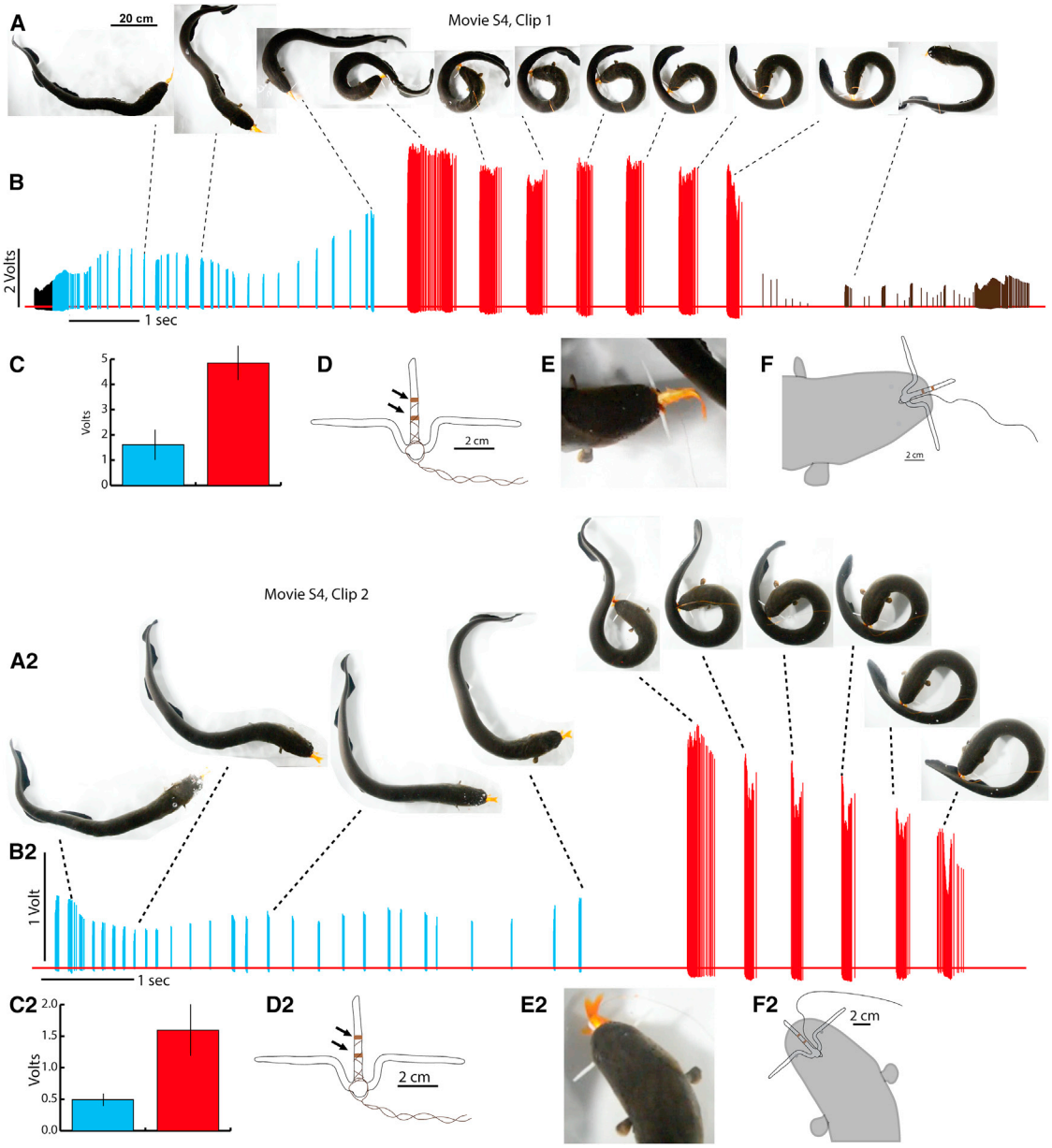

Figure 4. Experiment and Results for Measuring the Change in Field Strength within Prey during a Dipole Attack

Note that eels do not modulate their high-voltage amplitude, and thus voltage recordings can be primarily attributed to changes in field configuration.

(A) A large eel was presented with a pithed fish on a plastic holder with electrodes that could not be swallowed. After capture, the experimenter manually jiggled the wire to simulate prey struggling, and the eel curled to deliver multiple discharges.

(B) Voltages recorded from the electrode at different points during the eel's attack. Black tick marks indicate discharges before the eel firmly grasped the electrode preparation (left side) and after it repositioned the preparation (right side). Thus, those data were not used to compare relative voltages. Blue and red tick marks were all recorded while the eel held the electrode tightly but was either uncurled (blue) or curled (red). Note the dramatic increase in recorded voltage and discharge frequency during the curl relative to the uncurled configuration.

(C) Comparison of voltage between the two conditions (mean peak to peak voltage). Bars show the SD (t test significance $p < 0.0001$).

(D) Schematic illustration of electrodes with un-insulated wire (arrows) approximately 1 cm apart (see the Experimental Procedures).

(E) Closer view of an eel holding the electrode-fish preparation tightly.

(F) Schematic of electrode position during the trial.

(A2–F2) Conventions are as described above (C2, t test significance $p < 0.0001$).

See Movie S4, clips 1 and 2, for the two trials illustrated above.

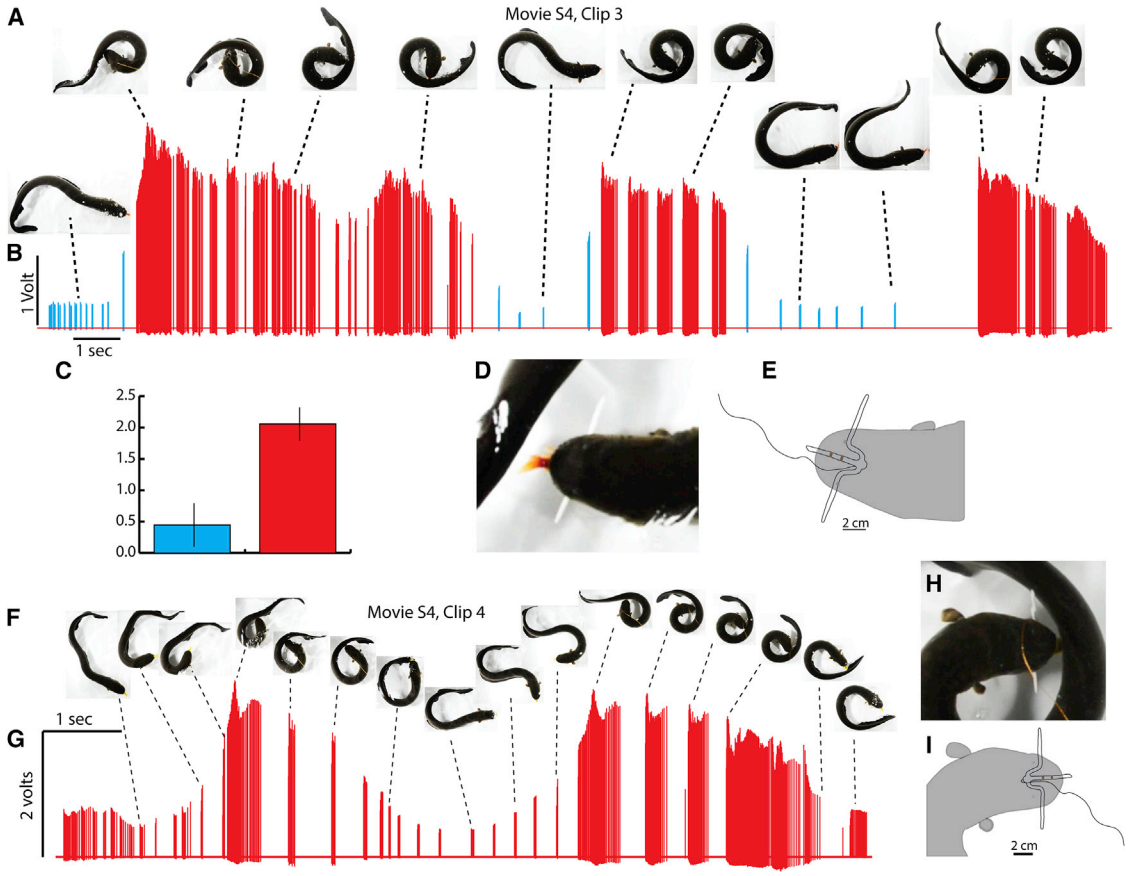

Figure 5. Additional Experiments and Results for Measuring the Change in Field Strength within Prey during a Dipole Attack

(A) Eel curled to deliver multiple discharges. Note the multiple curls and change in curl direction.

(B) Voltages recorded from the electrodes. Blue tick marks indicate uncurled configuration, and red tick marks indicate curled configuration.

(C) Comparison of voltage between conditions (mean peak to peak voltage). Curled and uncurled voltages were significantly different (t test significance $p < 0.0001$). Bars show the SD.

(D) Closer view of an eel holding the electrode-fish preparation.

(E) Schematic of electrode position during the trial.

(F–I) Conventions are as described above.

See Movie S4, clips 3 and 4, for the two trials illustrated above.

contractile force as a result of fatigue. Interestingly, transcutaneous stimulation of efferents is frequently used by clinicians to activate human skeletal muscle to enhance rehabilitation or to maintain strength after CNS injury. A frequently reported limitation of this procedure is the early onset of muscular fatigue relative to innate patterns of muscle activation [41, 42]. This is usually attributed to the greater susceptibility of large motor neurons, with large axons, to transcutaneous electrical activation. Such motor neurons activate large numbers of fast muscle fibers that are the most fatigable. Eel discharges may have a similar effect, reversing the order of motor neuron recruitment compared to innate patterns of activation [43].

The tension traces in Figure 7 are reminiscent of the results obtained when curare or alpha-bungarotoxin are added to a chick biventor preparation [44]. There is a precipitous decline in muscle tension that corresponds to the onset of high-frequency volleys associated with the curling behavior. The eel is driving the prey's muscles at roughly 100 Hz during these volleys. This is at least ten times the rate at which fish fast-twitch fibers are normally activated and equivalent to the motor neuron activation patterns of "superfast muscle" [45]. The fast-twitch fibers that drive prey escape do not have the specializations required for contraction at this speed.

The effects of the eel's curling behavior on the electric field and on prey muscles seem clear. But it is important to put this behavior in a larger context. Eels use this tactic when handling large and especially struggling prey that cannot be immediately swallowed. Presumably, this would occur most frequently when small eels with low power outputs handle typical prey (Movie S1) or when large eels handle prey with resistive epidermis (Movie S3). In either case, the unamplified high-voltage discharge may not reliably produce tetanus [3]. The particular challenge for the eel at this point stems from its limited prey handling options. It must release the prey, often repeatedly, to reposition it for swallowing. The very cue that elicits the curling behavior—prey movement—is an indicator that prey may escape when manipulated (such an escape event has been documented for weakly electric fish hunting cichlids in Lake Malawi [46]). The eel's

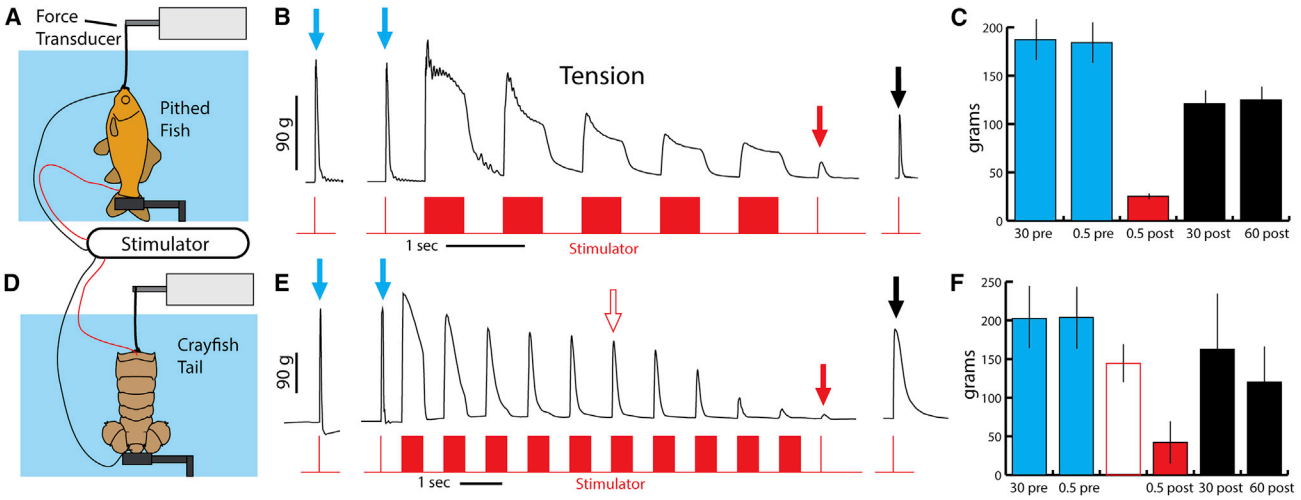

Figure 6. Paradigm Used to Simulate the Effect of Eel Volleys on Prey Muscle

(A) Pithed fish attached to a force transducer and stimulator.

(B) Example of whole-fish tension responses to single stimulator pulses prior to (blue arrows) a series of 500 ms, 100 Hz volleys, and after (red and black arrows) the volleys. Note the dramatic reduction on contractile force after five volleys (red arrow).

(C) Mean contractile force summed for ten different fish preparations. Blue represents mean contractile force for a single pulse 30 s and 500 ms prior to the volleys. The red column shows contractile force 500 ms after the last volley (eels juggle prey for swallowing within 500 ms of their last curled volley). Contractile force at this (red) time point was significantly different ($p < 0.001$, all comparisons) from other time points (ANOVA, degrees of freedom [df] = 4, $F = 16.93$, $p < 0.0001$, and Tukey's honest significant difference [HSD]). Black bars illustrate recovery of contractive force over time. Bars show the SE.

(D) Crayfish tail preparation and stimulator.

(E) Example of crayfish tail tension responses as described above. Note the difference in timescale and that more volleys (ten) were required to cause a similar reduction in contractile force. The unfilled red arrow indicates the time point corresponding to filled arrow in (B).

(F) Mean contractile force summed for five different crayfish tail preparations. Contractile force at 500 ms after the volley (filled red) was significantly different ($p < 0.02$, all comparisons) from other time points with the exception ($p < 0.07$) of after 60 s (black bar, far right) (ANOVA, df = 5, $F = 9.19$, $p < 0.0001$, and Tukey's HSD). The unfilled histogram represents the contractile force corresponding in time to the red histogram in (C). Bars show the SD.

solution is to greatly amplify the effect of the high-voltage discharge and remotely "over-activate" the prey neuromuscular system to produce fatigue. Repeated high-voltage volleys may have additional effects that attenuate prey movements. But remote, high-frequency activation of the most susceptible motor neuron efferents provides a reliable and apparently unavoidable mechanism for eels to temporarily incapacitate diverse prey.

From the standpoint of electrostatics and the basic physics of electric fields, it is not surprising that bringing the second pole of the electric organ around and behind prey amplifies the local field strength. Likewise, a long history of investigation of neuromuscular systems suggests repeated, high-frequency trains of motor neuron activation cause rapid muscle fatigue. Yet here the context of these observations is entirely unique, and it is remarkable that an animal has evolved the anatomical and behavioral traits to produce both of these effects.

EXPERIMENTAL PROCEDURES

All procedures were approved by the Vanderbilt Institutional Animal Care and Use Committee and followed the NIH guidelines for the care and use of laboratory animals. Eight eels (*Electrophorus electricus*) were housed in custom-made Plexiglas aquariums ranging in size from 80 to 120 gallons (300–480 l) with aerated water, gravel bottom, rocks, plastic imitation branches, and plastic plants; water temperature maintained between 24°C and 28°C; thermostatically controlled aquarium heaters; and a pH between 6.5 and 7.5. Lighting was on a 12 hr/12 hr light-dark cycle, and eels were fed earthworms, fish, and crayfish. Three larger eels included a 65 cm specimen, a 90 cm specimen, and a 120 cm specimen. The former were individually housed. In addition, five small, juvenile eels ranged in size from 10–15 cm. The latter were group housed.

Recordings of Eel Behavior

For recordings of juvenile eel hunting behavior, animals were transferred to custom made Plexiglas aquarium 40 cm × 100 cm and 20 cm deep with a translucent white bottom. Conductivity was maintained between 125 and 200 μS/cm. The electric organ discharges were recorded using carbon electrodes in the water connected on their exposed tips to wire leads from a split BNC cable that connected directly to one channel of either a PowerLab 8/35 or PowerLab 4/30 data acquisition unit (ADInstruments) sampling at a minimum of 100,000 per second and in turn connected to a MacBook Pro laptop running LabChart 7 software (ADInstruments). The aquarium was lit from below with two IR-Flood Ultra-Covert 940 nm illuminators (Night Vision Experts), and behavior was recorded with either a MotionPro HS-3 camera (Redlake) at 250 fps (slow motion) or a low-light charge-coupled device (CCD) camera (KT&C security camera) for real-time video. For recordings of larger eel behavior, eels were transferred to a 90 × 60 cm Plexiglas aquarium that was 30 cm deep and filled to a depth of 15–20 cm and were filmed with a Nikon D4 single-lens reflex (SLR) camera set to video mode using two RPS Studio CooLED 100 studio lights (RS-5610) for lighting. A Master 8 stimulator was used to activate visible diodes at the edge of the scene with the corresponding voltage output simultaneously recorded on the PowerLab 4/30 data acquisition unit. This allowed precise coordination of timing between real-time-recorded eel discharges and video of behavior. Coordination between the high-speed camera and lab chart recordings was through a dedicated time-marking voltage that signaled each frame in the separate channel. For illustration of the relationship of high-voltage electric organ discharge (EOD) to behavior in Movie S1, each frame during which an EOD occurred was colorized in Photoshop CS 6 (Adobe Systems). The tiff format image files were then

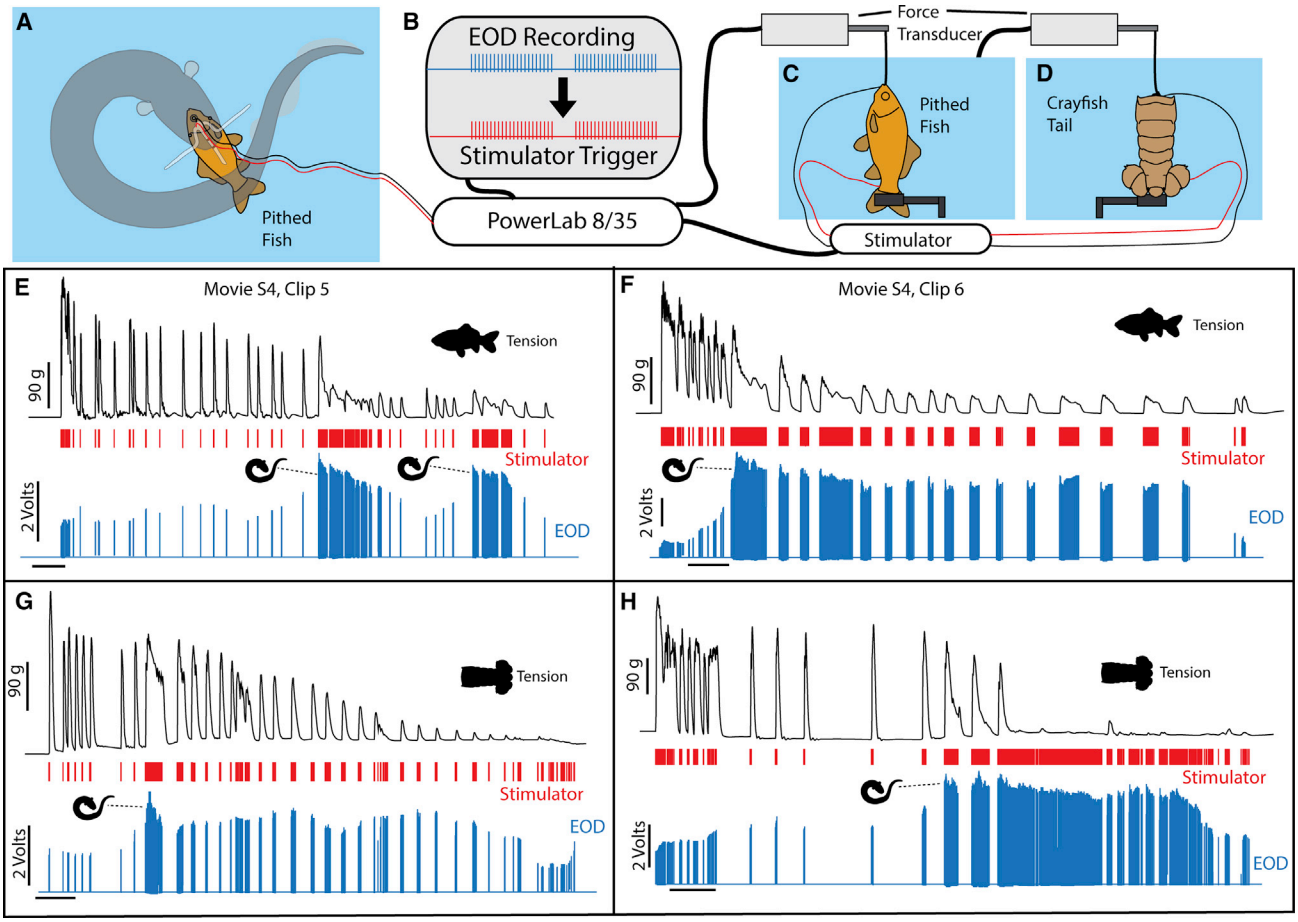

Figure 7. Paradigm Used to Stimulate Muscle Preparations with the Discharge Pattern of Eel Volleys

(A) An electric eel was induced to perform a curling attack on the prey-electrode preparation.

(B–D) The recorded high-voltage EOD triggered a Grass SD9 Stimulator (B) connected to either a pithed fish preparation (C) or a crayfish tail preparation (D) connected in turn to a force transducer.

(E–H) Tension, stimulator output, and electric eel EODs were simultaneously recorded (muscle preparation in the adjacent aquarium). Note that although eel discharges varied in amplitude (blue), increasing with curl as previously described, all stimulations were carried out at a fixed voltage. Tension in each preparation dropped dramatically over time, and particularly quickly when subjected to the continuous high-frequency stimulation that co-occurs with curling.

opened in sequence in QuickTime Player 7 Pro (Apple), and the sequence was exported as a QuickTime movie. Because low-light video was recorded at 250 fps, some colorized frames correspond to two discharges, and some gaps between discharges are not represented by uncolorized frames.

Measurement of Voltage in Pithed Fish

For investigation of the effect of the eel's curling on relative electric field strength, an electrode configuration was designed using InstaMorph moldable plastic (Happy Wire Dog) and 30G (large eels) or 36G (small eels) insulated motor winding wire (McMaster-Carr). Insulation from the distal portion of each of two wires was removed. These portions of wire were wrapped around the heat-softened extension of the moldable plastic holder at approximately 1 cm distance from one another. The insulated lengths of each wire were then sparsely wrapped along the remaining length of the holder and then braided together to form a single lead by which the electrode preparation could be attached to a BNC cord that was in turn connected to an input channel of the PowerLab data acquisition unit (see Figure 4D for an illustration of the electrode configuration for large eels, and Figure S1 for an illustration of the electrode configuration for small eels). A dead, pithed fish (e.g., [3]) with viable muscles was used as prey for these trials, and the long end of the electrode holder was inserted into the fish preparation as shown in Figures 4 and 5, and as seen in Movie S4. For illustration of the high-voltage output of the eel in relationship to eel behavior, data traces were copied at high-resolution from the LabChart 7 program into Adobe Illustrator, and each component was illustrated with vector graphics to allow appropriately scaling to variable final figure sizes.

Muscle Tension Measurements

Tension was measured in the pithed fish or crayfish tail preparation (the latter being removed after cold anesthesia and mechanical destruction of the rostral nerve cord) by securely clamping the caudal end of each preparation at the bottom of the water filled chamber and securing the rostral end to a MLTF500/ST force transducer (ADInstuments) using insulated motor wire with insulation removed at the site of attachment to the preparation. This wire served as both one electrical lead for the stimulator and as the mechanical connection to the force transducer. The other stimulator lead was incorporated into the plastic clamp that made contact with the preparation at the base. A Grass SD9 Stimulator was used to deliver the square wave (1 ms) stimuli at a setting of 40 V. The voltage produced within pithed fish at this setting was tested by inserting the previously described electrodes into a pithed fish situated in the transducer paradigm and recording the internal voltage produced by the stimulator. The stimulator produced just under 1 V as measured through the electrodes in the fish. For regimented, fixed-frequency stimulation as described and illustrated in Figure 6, the Grass SD9 Stimulator was in turn

driven by a Master 8 stimulator that was programmed to produce the desired output (Figure 6, red traces). The tension response and stimulator output were simultaneously recorded.

For measurement of tension produced by the pattern of pulses generated by an eel in real time (Figure 7), two PowerLab units were used. One unit recorded the eel EOD, and LabChart's "Fast Output Response" feature was used to produce a triggering output for each eel high-voltage EOD. The same PowerLab unit was coordinated with real-time video through the Nikon D4 as previously described using diodes and corresponding voltage recordings from the Master 8 stimulator. The leads recording the eel's EOD were split, and one set went to the second PowerLab unit connected to its own computer. This unit simultaneously recorded the stimulator output (Figure 7, red traces), the EOD amplitude (Figure 7, blue traces), and the muscle tension response (Figure 7, black traces). Data were illustrated as described above.

Voltages or tension magnitudes were measured by selecting the relevant area of the LabChart 7 trace and using the "min-max" measurement function and then using the Datapad function to collect and export the values to Microsoft Excel. Data were imported into the JMP statistical program (SAS), and the data were assessed with a t test or an ANOVA followed by Tukey's HSD as reported in the figure legends.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and four movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.09.036.

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